GenoSensor Corporation

GenoExplorer™ miRNA Biochips Kit

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Version B
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User Manual

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Literature Citation
When describing a procedure for publication using this product, we would appreciate that you refer to it as the GenoExplorer™ miRNA Biochips Kit.

Patents and Trademarks
GenoExplorer is a trademark of GenoSensor. The GenoExplorer™ miRNA Biochips Kit is covered by patents pending.

Product Overview

GenoExplorer™ Biochips

Comprehensive Probe Content

GenoExplorer™ chips consist of probes for all miRNAs as registered and annotated in the miRBase at The Wellcome Trust Sanger Institute. A key differentiator of the GenoExplorer™ chip's content is the inclusion of probes for precursor miRNA as well as mature miRNA. The content also includes probes for positive controls (*e.g.*, 5S rRNA, tRNAs, U6) as well as negative controls.

Customizable Chip Formats

GenoExplorer™ chip products come in several formats. Check our website for updates on the species-specific chips available. Orders of other species are also available upon request.

3-D Gel Matrix for Optimal Hybridization Environment

The surface of the GenoExplorer™ chip is composed of a 3-D gel matrix which provides a more optimal hybridization environment by extending the probe into the solution and increasing its access to the target.

Kit Components and Storage Conditions

GenoExplorer™ miRNA Full Kit (20 reactions)

Components	Amount	Storage
Array Chip	5 x 4 chips	+4º C
2x Hyb Buffer	5 x 280 µl	+4º C
Array Layout CD	1	
User's Manual	1	

Shipping and Storage

GenoExplorer™ biochips and hybridization reagents are shipped at room temperature. Components should be stored at temperatures shown in the above table. At proper storage conditions, components are stable for 1 year from the date received. Expiration dates are also noted on the tube labels of each component.

Safety Warnings and Precautions

For research use only. Not recommended or intended for the diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals. Consider all chemicals as potentially hazardous. Only persons trained in laboratory techniques and familiar with the principles of good laboratory practice should handle these products. Wear suitable protective clothing such as laboratory overalls, safety glasses, and gloves. Exercise caution to avoid contact with skin or eyes: if contact should occur, wash immediately with water (Material Safety Data Sheet for products is available upon request).

Additional Required Materials

Recommended: GenoExplorer™ miRNA labeling kit
Recommended: GenoExplorer™ reagents for hybridization assay
Total RNA containing the small RNA
RNase-free water
Adjustable pipettors
RNase-free tips
RNase-free polypropylene microcentrifuge tubes (0.2, 0.5 or 1.5 ml)
Graduated cylinder
Microcentrifuge
Incubator set at 37º C
Incubator or heating block set at 75º
Heating block at 95º C
Hybridization Station (optional), for automated hybridization (*e.g.*, Genomic Solutions, Tecan) **or**
Coverslips, for manual hybridization (*e.g.*, Erie LifterSlips™ , Product # 22x30I-2-4374)
Slide Chamber, for manual hybridization (*e.g.*, Corning® Microarray Hybridization Chamber, Product #2551)
Incubator or water bath set at 42º C, for manual hybridization
50 ml screw-top tubes, for manual post-hybridization and post-stain washing
Bottles for storing diluted Wash Buffers
Centrifuge or air blower
Microarray scanner and Image Processing Software

The GenoExplorer™ Biochips kit is open to a variety of equipment. The examples given are only suggestions rather than specific recommendations. Please contact technical support if you have specific questions.

Related Products from GenoSensor

GenoExplorer™ microRNA Full Kit
GenoExplorer™ microRNA Labeling kit
GenoExplorer™ microRNA Probe Set
GenoExplorer™ Reagents for Hybridization Assay

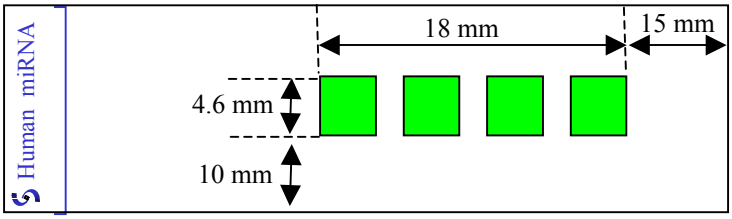
GenoExplorer™ miRNA Biochips Hybridization Assay Guidelines

General Description

GenoExplorer™ biochips are built on a 1" x 3" (25 mm x 75 mm) slide. The chips are coated with patented polymers used for DNA probe attachment. The biochips covalently immobilize amine-modified DNA oligonucleotide probes for use in microarray analysis. The chips demonstrate high probe attachment efficiency and accessibility of nucleic acid targets for hybridization.

Figure 1 shows the positions of the label and probe arrays on the slide. The four arrays are positioned lengthwise 15 mm from the end of the slide opposite the label and centered widthwise 10 mm from the side of the slide. The arrays area covers a length of 18 mm and a width of 4.6 mm.

Figure 1: Array positions on the chips



Biochip Handling and Assembly

Biochips are stored in a chip container. It is recommended to wear powder-free gloves when handling the Biochips. Open the container carefully to avoid dropping slides. Hold the slide at its edges and avoid touching the side of the slide containing the probe array. The probes are on the side of the slide which faces you when reading the slide label correctly (refer to figure 1). Prepare the chips for hybridization promptly to minimize possible accumulation of dust from the air on to the surface of the arrays (even if some dust can be washed away after post-hybridization washing procedures). Use assembly methods as recommended by hybstation or hybchamber manufacturers. For automated hybridization using a hybstation, orient the chips such that the probe side is up in the chamber and the slide-end opposite the label (where the arrays are located) are next to the sample injection port. Once the hybridization solution is introduced to the chip, avoid any drying of the chips until after the final wash step.

Assay Guidelines

The GenoExplorer™ miRNA Biochips Kit has been optimized for microarray experiments using GenoExplorer™ reagents for hybridization assay. Below are general guidelines that can be used for running the hybridization assay on GenoExplorer™ BioChips. For a detailed protocol on running GenoExplorer™ labeled miRNA on GenoExplorer™ BioChips, please consult the user manual for the GenoExplorer™ full kit.

1. Prepare the labeled RNA sample for hybridization by combining with 2x Hyb buffer and water. The volumes of each component to be added will depend on the total hybridization volume which is determined by the type of hyb chamber or coverslip that is being used. Table 2 shows the volumes of each component required for different total hybridization volumes.

Table 2: Hybridization Reaction Mix

Hybridation Volume (µl)	Labeled RNA (µl)	2x Hyb Buffer (µl)	Added H_2O (µl)
40	20	20	0
60	20	30	10
100	20	50	30
120	20	60	40

2. Incubate the sample at 90-95º C for 5 min.
3. Cool sample by spinning down for 2 min.
4. Load the labeled RNA on to the array. Consult with the slide chamber manufacturer's recommended protocol for further information on assembling the slide in its chamber.
5. Run the hybridization at 42º C for 16 hours (or longer).
6. For fluorescently-labeled RNA target, after hybridization the arrays can undergo post-hybridization washing and drying. GenoExplorer™ labeled wash buffers are strongly recommended for the post-hybridization wash processing. These buffers have been optimized to enhance signal and reduce background. For detailed instructions on the use of these buffers, consult the user manual for the GenoExplorer™ Full Kit.
7. For GenoExplorer™ labeled miRNA containing biotin labels, please refer to the user manual of the GenoExplorer™ Full Kit for further instructions on the post-hybridizaton process.

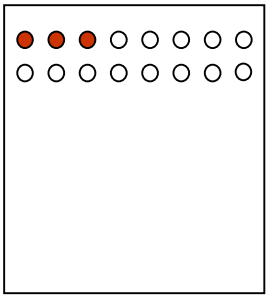
Scanning and Detection

Consult the scanner manufacturer instructions for laser and PMT settings.

Using the Grid Files

The enclosed CD provides an array list in .txt file and .gal file formats. Four arrays were printed on each chip. The name and location of each chemical probe is listed and labeled in the software. Each chemical was printed in triplicate. Arrays include known microRNA probes as well as both positive and negative controls. Load the .gal files or convert the text file to the correct format that your scanner requires. Since the first chemicals on each array are positive controls, you should see them on the upper-left corner of each array, and they are in triplicate. Move the array grid until it aligns with the positive controls in the upper-left corner (Figure 2). Proceed with data computation after gridding.

Figure 2: Grid and Align the Arrays



Appendix

Troubleshooting Guide

Poor Hybridization Signal

Poor quality RNA samples	• Use higher quality RNA samples • Use proper laboratory techniques when handling RNA samples • Label and hybridize more sample to microarray
Suboptimal amount of sample applied to microarray	• Label and hybridize more sample to microarray
Improper detection strategy	• Verify that detection instrumentation is compatible with detection reagents • Adjust detection settings
Signal lost by exposure to light, environmental conditions	• Minimize exposure to light
Poor biotin/ SA-S dye detection	• Ensure that the samples have been purified and quantified properly before labeling • Ensure that the kit components have been stored properly • Ensure that the detection solution was prepared and stored properly
Hybridization signal “stripped” from microarray	• Ensure correct temperatures are used for each of the process steps.
Suboptimal hybridization time	• Extend hybridization time

High Background

Excess detection reagents remaining on microarray	• Do not allow slides to dry out during hybridization protocol • Do not touch microarray directly or forcibly remove coverslip at any time
Excess sample applied to microarray	• Quantify the amount of labeled sample and use less in hybridization
Ink or marker used to identify microarray	• Avoid using markers or ink to identify slide; use a diamond scribe pen

Dust

Dust on microarray	• Avoid dust from environment • Minimize exposure of the microarray to the air • Use filtered distilled water for final wash

Technical Service

For more information or technical assistance, please call, write, fax, or email.

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Limited Warranty

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